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INACTIVATION ACTION SPECTRA OF *Bacillus subtilis* SPORES IN EXTENDED ULTRAVIOLET WAVELENGTHS (50–300 nm) OBTAINED WITH SYNCHROTRON RADIATION

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Abstract—Five types of *Bacillus subtilis* spores (UVR, UVS, UVP, RCE, and RCF) differing in repair and/or recombinational capabilities were exposed to monochromatic radiations at 13 wavelengths from 50 to 300 nm in vacuum. An improved biological irradiation system connected to a synchrotron radiation source was used to produce monochromatic UV radiation in this extended wavelength range with sufficient fluence to inactivate bacterial spores. From the survival curves obtained, the action spectra for the inactivation of the spores were depicted. Recombination-deficient RCE (*recE*) and RCF (*recF*) spores were more sensitive than the wild-type UVR spores in the entire range of wavelengths. This was considered to mean that DNA was the major target for the inactivation of the spores. Vacuum-UV radiations of 125–175 nm were effective in killing the spores, and distinct peaks of the sensitivity were seen with all types of the spores. Insensitivities at 190 and 100 nm were common to all five types of spores, indicating that these wavelengths were particularly impenetrant and absorbed by the outer layer materials. The vacuum-UV peaks centering at 150 nm were prominent in the spores defective in recombinational repair, while the far-UV peaks at around 235 and 270 nm were prominent in the UVS (*uvrA ssp*) and UVP (*uvrA ssp polA*) spores deficient in removal mechanisms of spore photoproducts. Thus, the profiles of the action spectra were explained by three factors; the penetration depth of each radiation in a spore, the efficiency of producing DNA damage that could cause inactivation, and the repair capacity of each type of spore.

INTRODUCTION

Biological effectiveness of UV radiation varies markedly, depending on the wavelengths used for the irradiation. This dependence is represented by an action spectrum in which the efficiency of producing a particular effect is plotted *vs* the wavelength of the radiation. This information is valuable in defining a photobiological response and in deducing the nature of the chromophore and target molecules causing the effects. In the analyses of cellular inactivation and mutagenesis, it has also been useful to use mutant strains defective in particular mechanisms of DNA repair. This has greatly helped in defining and identifying responsible DNA damage; for example, the formation of pyrimidine dimers is correlated to the cellular lethality in repair-deficient cells (Setlow, 1964; Webb and Brown, 1976). So far such works are concerned mostly with far and near UV wavelengths of 230–400 nm (Jagger, 1985).

The availability of synchrotron radiation for biological experiments enabled workers to extend the wavelengths to the vacuum-UV region (Ito *et al.*, 1984). However, as the wavelengths become shorter, absorption not only by biological materials but also by water or air becomes intense, and extremely high vacuum must be maintained throughout the beam line including irradiation chambers. A uni-cellular system particularly suited for such studies is a bacterial spore. We have performed irradiations with three types of *Bacillus subtilis* spores in the wavelengths from 150 to 250 nm, and found that, besides a prominent effect at 250 nm particularly with repair-deficient spores, the vacuum-UV wavelengths seemed efficient in causing lethality and mutation (Munakata *et al.*, 1986).

Since the effectiveness increased when the wavelengths were shortened, it was thought necessary to explore wavelengths shorter than 150 nm. Therefore, we have constructed an irradiation system operative below this wavelength. Major instrumental innovations have been made to remove the MgF_2 window between the monochromator and irradiation chamber, and the optical systems have been improved to increase the fluence rate (Hieda *et al.*, 1986; Saito *et al.*, 1988). The need to keep the chamber at high vacuum required a change of material to support samples, from a membrane filter to a glass plate. Also, the immense absorption and shielding by the sample have been circumvented by spreading spores at the lowest density possible.

Through these efforts, we have successfully obtained inactivation action spectra for five types of *B. subtilis* spores including two newly-employed recombination-deficient ones in an extended wavelength range of 50 to 300 nm. The results clearly

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demonstrated that vacuum UV radiation was effective in inactivating bacterial spores with peaks around 170–150 nm, which were magnified in recombination-deficient spores.

MATERIALS AND METHODS

Bacterial strains. Five strains of *B. subtilis* were used: HA101 (*uvr⁺ hisH101 metB101 leuA8*), TKJ6312 (*uvrA10 ssp-1 hisH101 metB101 leuA8*), TKJ6321 (*uvrA10 ssp-1 polA151 hisH101 metB101*), TKJ6411 (*recE4 thyA thyB hisH101 lys-21*), and TKJ6412 (*recF7 hisH101 lys-21*). The alleles of *recE4* and *recF7* originated from strains GSY908 and HA106, respectively (Harford, 1974). Strain HA101 was wild-type with regard to repair capabilities, while strains TKJ6312 and TKJ6321 were deficient in repair of UV-induced spore photoproducts. In addition, the latter was defective in DNA polymerase 1 (Munakata and Rupert, 1975; Munakata *et al.*, 1986). These three strains, HA101, TKJ6312 and TKJ6321, were termed UVR, UVS, and UVP*, respectively. The other two strains, TKJ6411 and TKJ6412, carried mutations causing deficiencies in recombination. The *recE* gene like the *recA* in *Escherichia coli*, was responsible for homologous recombination and for global response to DNA damage (Lovett and Roberts, 1985; Marrero and Yasbin, 1988). The amino acid sequence of the *recF* gene product was similar to that of the *recF* gene product in *E. coli* and the product was considered to function in a RecF pathway of recombination and repair (Moriya *et al.*, 1985; Smith, 1988). These two newly employed strains, TKJ6411 and TKJ6412, were termed RCE and RCF, respectively.

Samples for irradiation. The preparation of the spore sample dried on a membrane filter used in previous work (Munakata *et al.*, 1986) was found unsuitable for irradiation experiments with vacuum-UV radiations of wavelengths below 150 nm, since it was difficult to maintain high vacuum (below 10^{-5} Pa) in the presence of filter material, and shielding by overlapping spores or by membrane pores could not be eliminated. Therefore, the support material was changed to 5.5 × 20 mm glass plates (No. 5, Matsunami Glass Ind., Osaka). Purified spore suspension in 0.01% of aqueous solution of polyvinyl alcohol was stored at 2×10^7 per mL at 4°C. Five μL of the suspension containing 10^5 spores was spotted onto a glass plate in a rectangular area of 3 × 5 mm, the outside of which was made water-repellent with Polyflon (Daikin Ind., Osaka). Since the length of a spore is about 1 μm, the area was sufficiently large to hold 10^7 spores in a single layer. The sample was air-dried at 40°C and kept in a desiccator before use.

Irradiation system. The irradiation system has been installed at a beam line of an electron storage ring at the Institute for Solid State Physics (University of Tokyo) at Tanashi-shi, Tokyo. The 0.38 GeV storage ring was operated at a maximum current of *ca* 350 mA with a half life of *ca* 2 h. A Wadsworth-type monochromator covered the entire wavelength range from 50 to 300 nm. Two irradiation chambers were used; the same one as used previously for 150–250 nm irradiations (Munakata *et al.*, 1986) and an improved construction to allow the removal of the MgF_2 window in front of the chamber. In the latter case, an optical system made of Au-coated grating and mirrors was employed (Hieda *et al.*, 1986). In both chambers, a sample holder could accommodate six glass plates. Uniform fluence rates were obtained in an area of 5 × 7 mm that covered the entire area (3 × 5 mm) with spores. The half-maximum band width was estimated to be less than 5 nm in the entire range (Ito *et al.*, 1984). The fluence rates were determined on each operating day for the entire wavelength range with salicylate fluorescence, and this was standardized at 255 nm with a standard photon counter as described before (Ito *et al.*, 1983). The fluence rates (photon/m²/min) at the sample position were proportional to the ring current and ranged from 1×10^{19} (50 nm) to 5×10^{19} (220 nm) when the ring current was 200 mA.

Irradiation procedure. The sample holder with six glass plates and additional slots for control samples was inserted in a holder slot of the irradiation chambers. The chambers were evacuated to below 10^{-5} Pa for 50–135 nm irradiation or to below 10^{-3} Pa for 150–300 nm irradiation before the light path was opened. The exposure was started by opening a shutter placed in front of the monochromator and was regulated by a fluence-controlled program that operated according to the integration of the ring current and the time. The samples were changed by a knob from outside. When all the six samples were irradiated, the vacuum in the chamber was broken, and the samples were recovered in the air.

Recovery of the spore and the assay of survival. Irradiated spores were recovered by suspending each glass plate in 1 mL water. This suspension was sonicated by a cell disrupter (Kontes, Vineland, NJ) with a 10 cm probe for 5 s, and this was heated at 75°C for 15 min. Appropriate portions of the suspension were plated on an agar medium consisting of Spizizen minimal with the addition of 50 mg/L casein hydrolysate enzymatic (ICN Pharmaceutical, Cleveland, OH) and required nutrients (Munakata *et al.*, 1986). We detected no loss of viability by exposure to vacuum up to several hours for UVR, UVS or UVP spores (more than 20 samples for each strain were assayed). But for RCE or RCF spores, exposure to extremely high vacuum sometimes reduced the viability by up to 40%. Therefore, for these spores, control samples were always placed in the same chamber and fractional survivals were obtained relative to the controls.

Analysis of survival curves. Survivals were determined for each of the five types of spores at 13 wavelengths and plotted against photon fluences. The survival (N/N_0) was assumed to decline exponentially dependent on the photon fluence (F). The plot was fitted to $N/N_0 = a\exp(-kF)$ where the y-axis intercept (a) and the slope of the curve representing the inactivation rate constant (k) were obtained by linear regression of the fractional survival on the photon fluence.

RESULTS

Survival curves

Survival curves were obtained for five types of spores at 13 wavelengths from 50 to 300 nm (Fig. 1). Inspection of the data points revealed that for all types of spores, survival declined exponentially with increasing photon fluence at least to 5% at all wavelengths, including the shortest one. This means that shielding due to overlap of the spores was negligible. It was also evident that there were no notable differences in patterns of surviving curves among the different types of spores or different wavelengths used. Therefore, it was assumed that the survival data could be fitted to a simple exponential relationship defined by a y-axis intercept (a) and a slope (k) representing an inactivation rate constant. The calculated values obtained from regression analyses

*Abbreviations: UVR, UVS, UVP, RCE, and RCF, the strains HA101, TKJ6312, TKJ6321, TKJ6411 and TKJ6412, respectively.

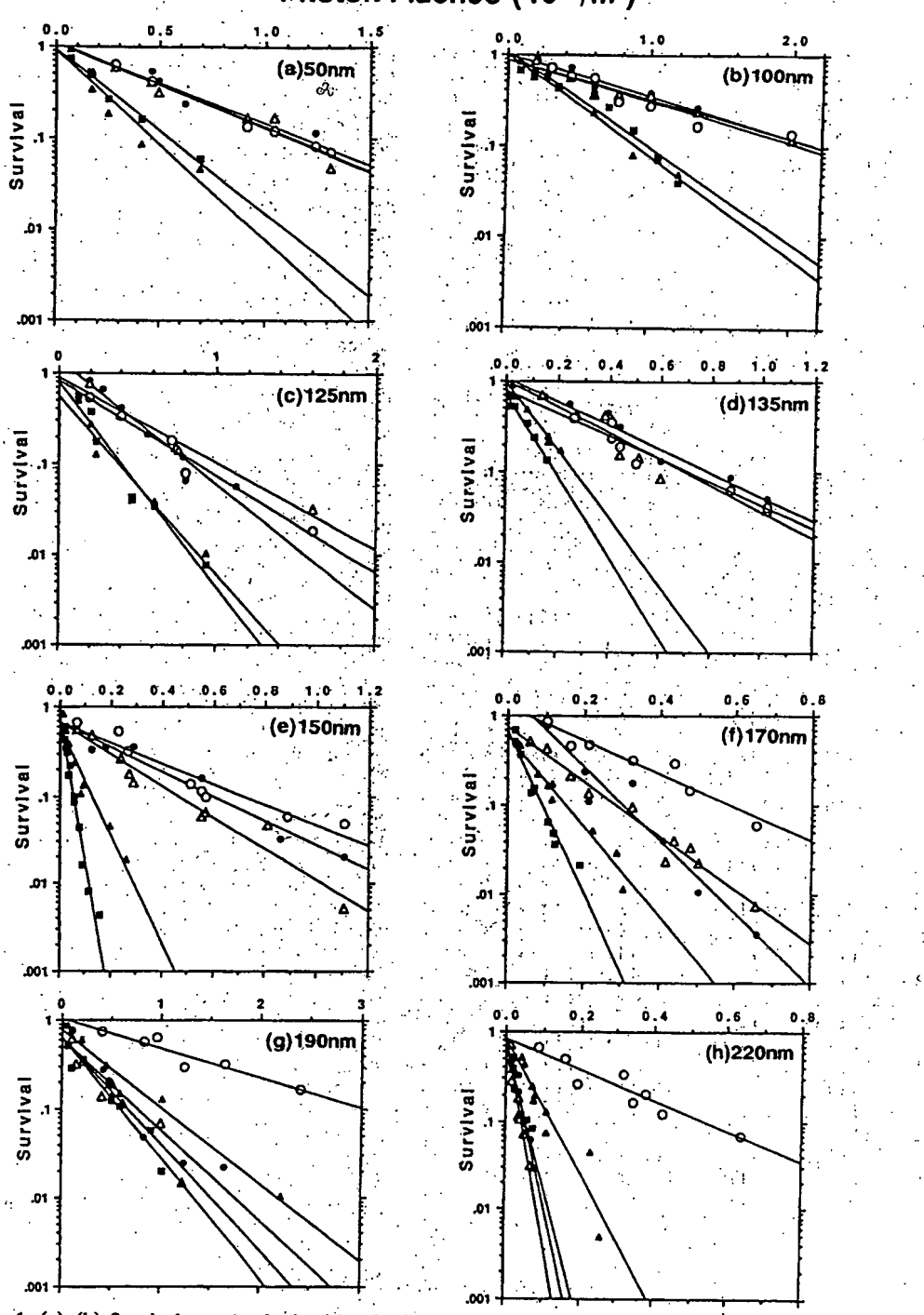

Figure 1. (a)–(h) Survival curves obtained by the irradiation of five types of *B. subtilis* spores at 13 wavelengths. Strain spores: UVS (●), UVP (△), UVR (○), RCE (■), and RCF (▲) (*continued overleaf*).

were used to draw straight lines in the figures. In most cases, the y-axis intercept is close to 1.0, showing an exponential loss of survival without a significant shoulder. Since the means of the y-axis intercept for each type of spore and for each wavelength are in the one standard deviation (SD) range of the total mean (0.95 ± 0.35), it was considered that this value did not significantly depend on strains or wavelengths. Though it was possible that some fine features of survival curves were missed due to the scatters of the data points, we concluded that the variations of the a-values were insignificant. This left us with the inactivation rate constant (k) as the sole parameter to be used as the indicator of

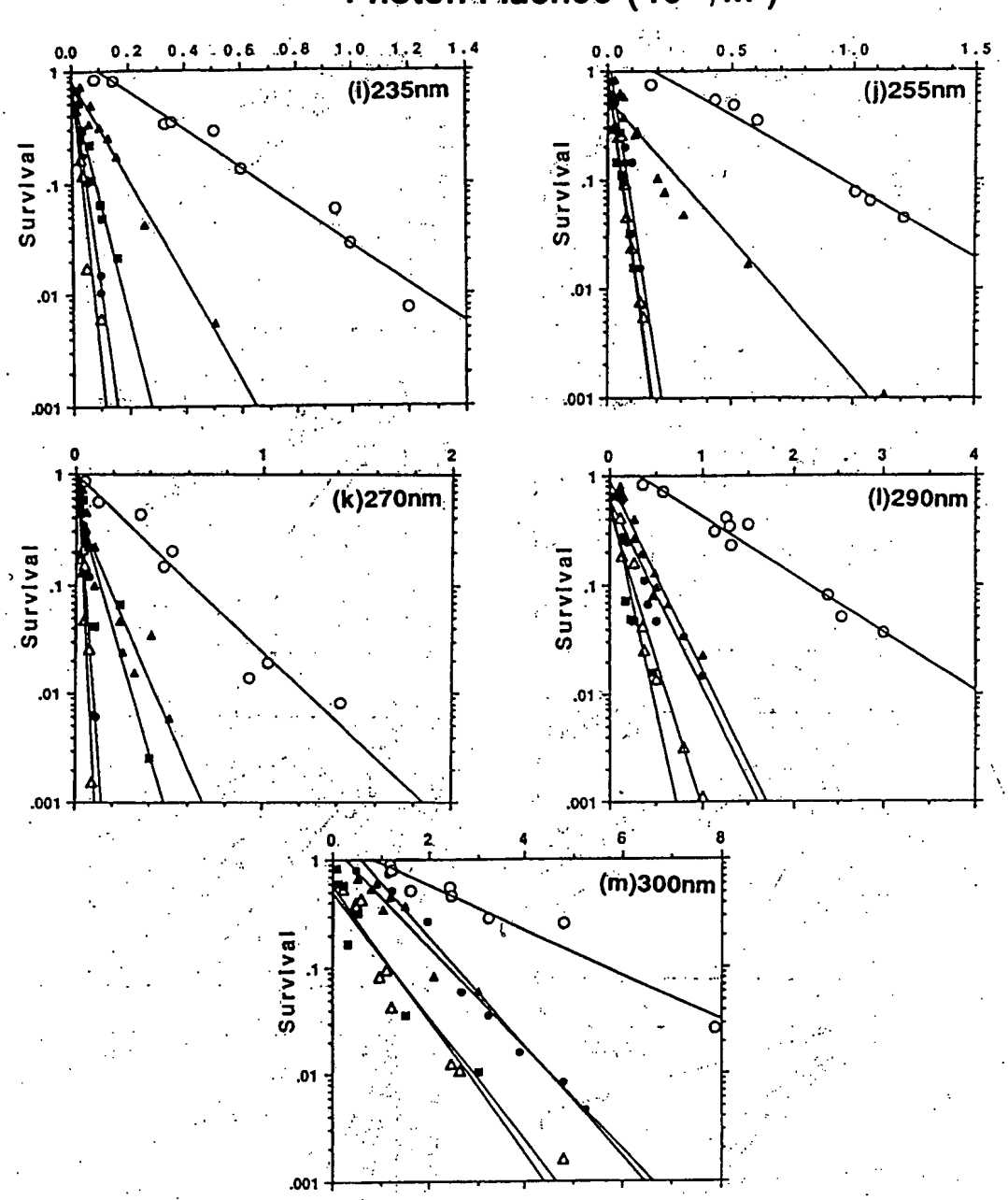

Figure 1. (i)–(m) Survival curves obtained by the irradiation of five types of *B. subtilis* spores at 13 wavelengths. Strain spores: UVS (●), UVP (△), UVR (○), RCE (■), and RCF (▲).

sensitivity of the spore and effectiveness of the radiation. The values of k with the standard error of the estimate of the slope are summarized in Table 1 and they form the bases of the subsequent treatments and discussion.

Action spectra

The values of the inactivation rate constant (k) that are listed in Table 1 were used to draw the action spectra for the five types of spores (Fig. 2). Since these values cover a range of more than two orders of magnitude, they are plotted in a logarithmic scale in the figure. There are several features that are common to all five types of spores. As the wavelengths are shortened from 300 nm, the efficiency for inactivation increases until it reaches a peak at around 270–255 nm. This is followed by a dip and another peak at 235–220 nm. It declines drastically at 190 nm and then recovers to form a peak at 170–150 nm. It then decreases steadily to 100 nm, and turns to increase gradually to 50 nm.

Table 1. Summary of survival curves. Values of the y-axis intercept (a) and the slope representing the inactivation rate constant (k) with the standard error (SE) are calculated from exponential regression of fractional survival on photon fluence ($10^{20}/m^2$)

Wavelength (nm)	UVS		UVP		UVR		RCE		RCF	
	a	k (SE)	a	k (SE)	a	k (SE)	a	k (SE)	a	k (SE)
50	1.08	2.02 (0.25)	1.08	2.17 (0.28)	1.11	2.15 (0.20)	0.93	4.13 (0.27)	0.89	4.74 (0.78)
100	1.01	1.07 (0.10)	0.87	1.05 (0.11)	0.87	1.05 (0.16)	1.08	2.42 (0.23)	1.01	2.58 (0.12)
125	1.41	3.16 (0.39)	0.88	2.15 (0.22)	0.82	2.40 (0.27)	0.80	5.17 (0.51)	0.56	4.48 (0.61)
135	1.07	3.03 (0.32)	1.01	3.33 (0.42)	0.78	2.91 (0.37)	0.70	10.2 (0.50)	0.96	8.58 (0.60)
150	0.67	3.19 (0.22)	0.66	4.08 (0.37)	0.67	2.69 (0.87)	0.73	36.5 (2.2)	0.60	13.9 (1.6)
170	1.79	9.48 (0.97)	0.76	7.03 (0.44)	1.24	4.26 (0.55)	0.70	21.0 (1.8)	0.61	11.7 (1.1)
190	0.63	2.37 (0.36)	0.64	2.75 (0.33)	1.05	0.76 (0.14)	0.65	3.13 (0.32)	0.78	2.00 (0.16)
220	0.85	41.6 (6.0)	0.90	52.6 (5.5)	0.82	4.00 (0.61)	0.69	36.0 (5.3)	0.83	17.3 (2.4)
235	0.92	43.8 (1.2)	0.94	58.6 (7.9)	1.43	3.95 (0.29)	0.71	23.5 (1.6)	0.83	10.2 (0.50)
255	1.46	32.8 (3.8)	1.12	37.2 (2.4)	1.74	3.00 (0.23)	0.93	37.6 (3.5)	0.56	5.90 (0.41)
270	1.63	51.9 (6.3)	1.78	67.4 (8.7)	1.01	3.74 (0.36)	0.59	13.2 (1.7)	0.49	9.05 (1.27)
290	0.61	3.96 (0.51)	0.50	6.30 (0.53)	1.51	1.24 (0.09)	0.68	9.04 (1.75)	0.92	3.99 (0.43)
300	1.98	1.18 (0.07)	0.51	1.33 (0.14)	1.51	0.48 (0.05)	0.58	1.45 (0.21)	1.34	1.09 (0.14)

The UVS and UVP spectra generally follow this pattern with enhanced peaks in the far-UV region. The two recombination-deficient strains exhibit some interesting modulations when being compared to the UVR pattern; the peaks and troughs in far-UV wavelengths are shifted and, in vacuum UV wavelengths, greatly enhanced peaks are seen at 150 nm.

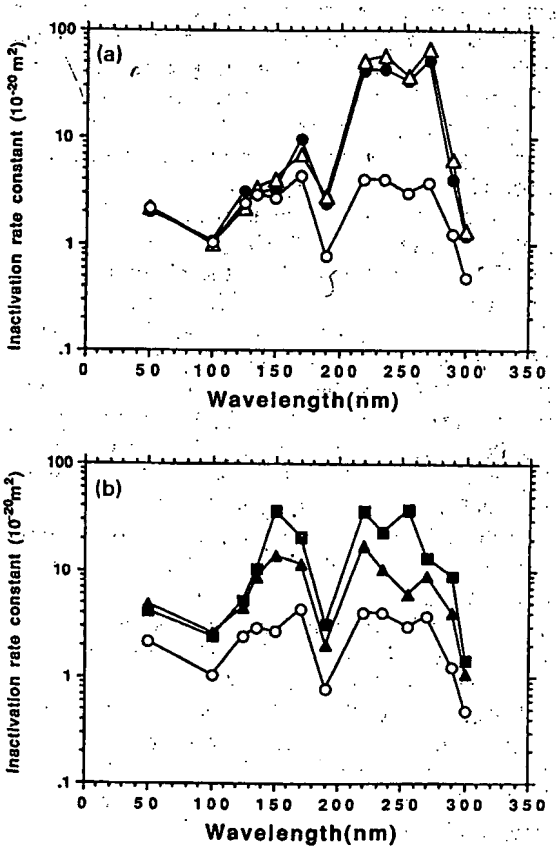

Figure 2. (a) Inactivation action spectra for the strain spores: UVS (●), UVP (△), and UVR (○). Inactivation rate constants (10^{-20}m^2) are plotted *vs* wavelengths. (b) Inactivation action spectra for the strain spores: RCE (■), and RCF (▲). The spectrum for the spores of UVR (○) is redrawn from Fig. 2(a) for comparison.

Comparison of action spectra between different strains

To demonstrate the extent of the differential sensitivities among the five types of spores, ratios of the inactivation rate constants of four types of the repair-defective strains to those of the repair-proficient UVR spores are calculated and shown in Fig. 3. The sensitivities of three types of spores, UVR, UVS, and UVP, are practically indistinguishable for wavelengths between 50 and 150 nm, thus the ratios are close to 1.0. These ratios increase as the wavelengths become longer and exhibit more than an order of magnitude from 220 to 270 nm. Two peaks at 235 and 270 nm and a trough at 255 nm are evident in both the absolute sensitivities of UVS and UVP spores and the ratios to UVR spores. On the other hand, two recombination-deficient strains (RCE and RCF) exhibit unique patterns. These two types of spores show significantly higher sensitivities than UVR spores in the whole range of wavelengths examined. At the shortest end, both are about 2-fold more sensitive than UVR. The sensitivities then increase sharply at around 150 nm. Then, for RCE, the next peaks are found in the far-UV range at 220 and 255 nm with a sharp decline at 270 nm. Also, the sensitivity is several times higher than UVR in the near-UV range at 290 and 300 nm, while, for RCF spores, minor peaks of sensitivity in far-UV and near-UV are seen at 220 and 290 nm, respectively.

These two types of spectra are summarized as follows. The wavelength range in question can be divided into three parts marked by prominent depressions at 100 and 190 nm observable for all five types of the spores. At 50 nm, three types of spores, UVR, UVS, and UVP, exhibit similar sensitivities, while RCE and RCF spores are about

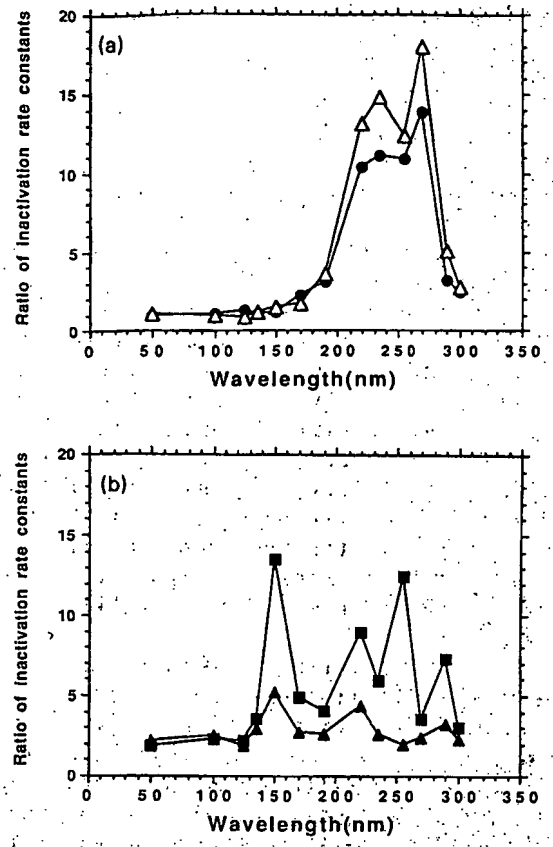

Figure 3. (a) Ratio of the inactivation rate constants of UVS (●) and UVP (△) spores to UVR spores. (b) Ratio of the inactivation rate constants of RCE (■) and RCF (▲) spores to UVR spores.

twice as sensitive as the other three. In the next region, between 100 and 190 nm, one prominent peak at 150 nm is seen for RCE and RCF. Also, UVS and UVP spores show moderate elevation and the ratios to UVR increase gradually as the wavelengths lengthen. In the far-UV range of 190–300 nm, UVR spores show an elevation with a minor depression at 255 nm and highest peaks of comparable magnitude at 220 and 270 nm. For UVS and UVP spores, these peaks are much more pronounced. The ratios to the UVR sensitivities also exhibit peaks at 235 and 270 nm. These two types of spores are thus 10–20 times more sensitive to far-UV wavelengths under these experimental conditions. The ratios of the sensitivities of RCE and RCF spores to those of UVR exhibit different profiles; for RCF spores, only a peak at 220 nm is significantly elevated. On the other hand, RCE spores exhibit three peaks at 220, 255 and 290 nm.

DISCUSSION

First, we focus on the features of the inactivation action spectra that are common to all five types of the spores. The spectra exhibit two depressions of the inactivation rate constant at around 190 and

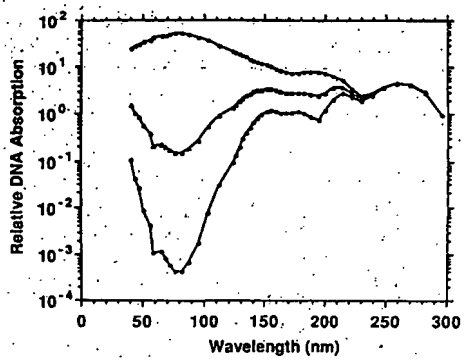

Figure 4. Hypothetical absorption spectra of DNA covered by a layer of albumin with thickness of 0 μm (○), 0.05 μm (▲), or 0.1 μm (●) calculated according to the absorption spectra of DNA (Inagaki *et al.*, 1975) and albumin (Inagaki *et al.*, 1974). At each wavelength, the absorption coefficient (μm^{-1}) of DNA is multiplied by the transmission (I/I_0) of the albumin layer.

100 nm. The insensitivity at 190 nm is characteristic and has also been demonstrated in previous work (Munakata *et al.*, 1986). We discussed the possibility that the strong attenuation of incoming radiation by proteinous outer layers of a spore as the cause of this ineffectiveness. This suggestion may also be extended to the depression at around 100 nm, since the absorption coefficients of organic materials, including proteins and DNA, exhibit broad maxima at around 80 nm (Inagaki *et al.*, 1974, 1975). We tried to simulate the profile of the action spectra by estimating the absorption to DNA when it is covered by a protein layer of a fixed thickness. This is done by multiplying the absorption coefficient of DNA (Inagaki *et al.*, 1975) at each wavelength with the value of transmission of the albumin layer of thickness 0.05 μm or 0.1 μm (Inagaki *et al.*, 1974). As seen in Fig. 4, the profile in wavelengths below 150 nm may be well simulated by the assumption that the DNA in the spore core is covered by a protein layer about 0.05 μm thick. The ratio of the sensitivity at 150 nm to that at 100 nm was about 15 for RCE spores. This value is attained by assuming a protein layer of 0.064 μm. Below 100 nm, the sensitivity increases slightly at 50 nm for all types of spores. This could be due to the increase in the efficiency of producing DNA damage as seen from the efficiencies of inducing strand breaks of DNA (Suzuki and Hieda, 1986) and of the inactivation of bacteriophage (Maezawa *et al.*, 1987).

Between these depressions, two main regions of high sensitivity are observed; one in the vacuum-UV at 150–170 nm and the other in the far-UV at 220–270 nm. These peaks of sensitivity exhibit unique differences among the five types of spores. We have pointed out in previous studies that, since mutagenic and lethal action spectra paralleled in the range 150–250 nm, these effects must be primarily caused by DNA damage. In the present work, we could not determine the mutagenic effectiveness

because the amount of spores irradiated was limited. However, the higher sensitivities for RCE and RCF than for UVS, UVP, and UVR, in the range of 150–50 nm suggest that at these wavelengths, too, DNA is the main target for inactivation. Interestingly, the differential sensitivities between RCE and RCF become very small at this short end of wavelengths (125–50 nm) compared to the longer wavelength region, where generally RCE spores are significantly more sensitive than RCF. This may mean that, in the shorter region, the nature of DNA damage is such that repair is predominantly by a pathway controlled by the *recF* gene product. In contrast, at wavelengths longer than 150 nm, damage repair is also dependent on *recE* gene product.

The most significant result obtained in this work is that vacuum-UV radiation of 135–170 nm exerts a potent killing effect. Though this vacuum-UV peak can be seen even with the wild-type (UVR) spores, it is more clearly marked with the *recF* and *recE* mutant spores. Photons in this wavelength range could be absorbed by DNA bases and cause ionization (Orlov *et al.*, 1976). However, it may be more likely that the absorption by DNA leads to superexcitation with subsequent fragmentation (Iwanami and Oda, 1983). This process may produce unique DNA damage subject to the repair pathway that depends on the proficiency of recombination. In contrast, in shorter wavelengths (125–50 nm), the more energetic photons are primarily absorbed by DNA sugar and lead to ionization producing DNA damage that is less well handled by the repair pathway.

In the far-UV region, the spectra for UVR, UVS, and UVP spores are concurrent with the previous observation (Munakata *et al.*, 1986). It is also clear in this work that the sensitivities peak at 220–235 nm and at 270 nm, forming a small trough at 255 nm. These two peaks are also produced when the UVS and UVP sensitivities are viewed in ratio to the UVR sensitivity. Thus, it seems that the nature of the DNA damage with regard to repairability is uniform in the far-UV range. Since it has been well documented that thyminyldihydrothymine-type spore photoproducts involving two thymidine residues are major products induced with far-UV irradiation of spores, and that UVS and UVP spores are defective in repairing such products, it is likely that these patterns of the spectra represent the efficiency of the formation of the spore photoproducts (Munakata and Rupert, 1974; Tyrrell, 1978). On the other hand, the newly-employed recombination-defective spores (RCE and RCF) exhibited somewhat different patterns. RCF spores, which show modest sensitivity in this region, are most effectively inactivated at 220 nm. In contrast, RCE spores are highly sensitive, almost as sensitive as UVS or UVP spores, but the two peaks are in the shorter wavelengths than those of UVS or UVP spores, forming troughs at 235 and 270 nm. The reason for these shifts in the fine structures of the action spectra is not known. It is possible that different types of DNA damage that are repaired preferentially by recombinational mechanisms are involved. Another possibility is that the presence of other constituents such as dipicolinic acid in the vicinity of DNA may affect the spectra by facilitating energy transfer in the unique environment of the spore core. This is supported by the fact that dipicolinic acid exhibits a closely parallel pattern of absorption spectrum in this wavelength region (Bailey *et al.*, 1965). The amount or distribution of this material may be somewhat different in the recombination-defective spores, particularly in RCE spores. These possibilities require further substantiation. The ratio of the sensitivity of UVS to UVR spores becomes smaller and less evident at 300 nm. This is unique for the vacuum irradiation, since it is known that UVS spores are about 20 times more sensitive than UVR spores when the irradiation is performed in air at 313 nm (Munakata, 1981).

Previously, the action spectra for inactivation and mutagenesis have been obtained in the 150–250 nm range for UVR, UVS, and UVP spores (Munakata *et al.*, 1986). The profiles of the inactivation spectra for each type of spore are similar to those presented above in this wavelength range. However, we note here that the absolute sensitivity seems somewhat lower in the present data. For example, previous values of the inactivation rate constants for UVR spores were 7.76, 1.30 and 6.47 ($\times 10^{-20}$ m^2) at 150, 190 and 250 nm, respectively, while in the current work, they are 2.69, 0.76 and 4.00 ($\times 10^{-20}$ m^2), respectively. Since the major change made in the experimental protocol is the use of glass plates for the support material instead of membrane filters, we have determined the difference in the sensitivity of these two different sample conditions under a germicidal lamp (254 nm) in air, and it was found that the sensitivity is about 2.0 times higher with the samples on membrane filters than those on glass plates (data not shown). Similar phenomena with a variety of biological specimens have been described before (Shinohara *et al.*, 1983). The exact cause of this effect is not known, but possibly related to the optical and geometrical properties of the support materials.

Since it is likely that solar radiation was more penetrant on the early earth due to the scarcity of oxygen in the atmosphere, far-UV and more energetic vacuum-UV radiation might have played important roles in the formation and subsequent evolution of primitive life. In these aspects, DNA repair mechanisms dependent on recombination capability might have been instrumental and indispensable. Experiments in a space environment will complement this work and improve understanding of its environmental and evolutional significance (Horneck *et al.*, 1984). It is also intriguing to extend

the present work to shorter wavelengths and to link it with the studies carried out using soft-x radiations (Munakata *et al.*, 1989). We are currently undertaking efforts to attain this goal.

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